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L5	l1 and L4	4	L5
L4	(hypoxia-inducible adj factor or hif) near6 (bind$ adj site)	23	L4
L3	l1 and L2	32	L3
L2	(viral or sv40 or mlv or mmlv or cmv) near3 (promoter or promotor or regulatory adj (sequence or element))	13507	L2
L1	hypoxia adj response adj element	39	L1

END OF SEARCH HISTORY

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Search Results - Record(s) 1 through 20 of 32 returned.

☐ 1. 20020192634 . 19 Dec 01. 19 Dec 02. EG-VEGF nucleic acids and polypeptides and methods of use. Ferrara, Napoleone, et al. 435/4; 435/6 435/69.5 C12Q001/00.

☐ 2. 20020173006 . 13 May 02. 21 Nov 02. Poly zinc finger proteins with improved linkers. Kim, Jin-Soo, et al. 435/69.1; 435/226 435/320.1 435/325 435/455 536/23.2 C12P021/02 C12N005/06 C12N009/64 C07H021/04 C12N015/87.

☐ 3. 20020172678 . 20 Jun 01. 21 Nov 02. EG-VEGF nucleic acids and polypeptides and methods of use. Ferrara, Napoleone, et al. 424/145.1; 514/12 A61K039/395 A61K038/18.

☐ 4. 20020169138 . 11 Feb 02. 14 Nov 02. Delivery vehicles for bioactive agents and uses thereof. Kunz, Lawrence L., et al. 514/44; 514/26 A61K048/00 A61K031/704.

☐ 5. 20020164575 . 28 Aug 01. 07 Nov 02. Gene identification. Case, Casey C., et al. 435/4; 435/6 C12Q001/00 C12Q001/68.

☐ 6. 20020160940 . 28 Aug 01. 31 Oct 02. Modulation of endogenous gene expression in cells. Case, Casey C., et al. 514/6; 435/455 A61K038/48.

☐ 7. 20020146691 . 06 Dec 00. 10 Oct 02. Methods of using randomized libraries of zinc finger proteins for the identification of gene function. Case, Casey C., et al. 435/6; 435/4 435/455 C12Q001/68 C12Q001/00 C12N015/87.

☐ 8. 20020127559 . 27 Apr 01. 12 Sep 02. Pharmacogenomics and identification of drug targets by reconstruction of signal transduction pathways based on sequences of accessible regions. Wolffe, Alan, et al. 435/6; 435/91.2 C12Q001/68 C12P019/34.

☐ 9. 20020120948 . 20 Sep 01. 29 Aug 02. Methods for expressing gene products. Fisher, Amanda. 800/3; 424/85.1 424/93.21 A61K048/00 G01N033/00 A61K038/19.

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☐ 11. 20020094968 . 28 Sep 01. 18 Jul 02. Nuclear reprogramming using IWSI and related chromatin remodeling ATPases. Wolffe, Alan P., et al. 514/44; 435/455 A61K048/00 C12N015/87.

☐ 12. 20020094529 . 28 Aug 01. 18 Jul 02. Gene identification. Case, Casey C., et al. 435/6; 435/4 435/455 C12Q001/68 C12Q001/00.

☐ 13. 20020081614 . 09 Aug 01. 27 Jun 02. Functional genomics using zinc finger proteins. Case, Casey C., et al. 435/6; 435/7.21 702/19 C12Q001/68 G01N033/567 G06F019/00 G01N033/48 G01N033/50.

☐ 14. 20020081603 . 27 Apr 01. 27 Jun 02. Databases of regulatory sequences; methods of making

and using same. Wolffe, Alan, et al. 435/6; 435/91.2 C12Q001/68 C12P019/34.

☐ 15. 20020076711 . 27 Apr 01. 20 Jun 02. Methods for designing exogenous regulatory molecules. Wolffe, Alan, et al. 435/6; 435/91.2 702/20 C12Q001/68 G06F019/00 G01N033/48 G01N033/50 C12P019/34.

☐ 16. 20020061294 . 05 Apr 99. 23 May 02. MONONUCLEAR PHAGOCYTES IN THERAPEUTIC DRUG DELIVERY. LEWIS, CLAIRE E., et al. 424/93.21; 424/450 435/320.1 435/325 435/69.1 514/2 514/44 A61K048/00 C12P021/02 C12N005/08 A61K038/17.

☐ 17. 20020048794 . 07 Aug 01. 25 Apr 02. Mechanism of conditional regulation of the hypoxia-inducible factor-1 by the von Hippel-Lindau tumor suppressor protein. Poellinger, Lorenz, et al. 435/69.1; 435/320.1 435/325 514/12 530/350 536/23.5 A61K038/17 C07H021/04 C07K014/435 C12P021/02 C12N005/06.

☐ 18. 20020045158 . 08 Feb 01. 18 Apr 02. Cells for drug discovery. Case, Casey. 435/4; 435/325 C12Q001/00 C12N005/06.

☐ 19. 20020019350 . 12 Feb 01. 14 Feb 02. Targeted angiogenesis. Levine, Arnold J., et al. 514/12; 530/399 A61K038/18.

☐ 20. 20010053352 . 09 Sep 99. 20 Dec 01. ADENOVIRUS VECTORS CONTAINING CELL STATUS-SPECIFIC RESPONSE ELEMENTS AND METHODS OF USE THEREOF. YU, DE CHAO, et al. 424/93.6; 435/320.1 435/69.1 514/44 536/23.5 A61K048/00 C12N015/861 C12P021/02 C07H021/04.

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Terms	Documents
l1 and L2	32

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☐ 21. 6511808 . 27 Apr 01; 28 Jan 03. Methods for designing exogenous regulatory molecules. Wolffe; Alan, et al. 435/6;. C12Q001/68.

☐ 22. 6503717 . 06 Dec 00; 07 Jan 03. Methods of using randomized libraries of zinc finger proteins for the identification of gene function. Case; Casey C., et al. 435/6; 435/320.1 435/455 536/23.5. C12Q001/68 C12N005/16 C12N015/12 C12N015/63.

☐ 23. 6479626 . 01 Mar 99; 12 Nov 02. Poly zinc finger proteins with improved linkers. Kim; Jin-Soo, et al. 530/300; 435/69.7 530/324 530/350. C07K002/00.

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☐ 25. 6410248 . 29 Jan 99; 25 Jun 02. General strategy for selecting high-affinity zinc finger proteins for diverse DNA target sites. Greisman; Harvey A., et al. 435/7.2; 435/4 435/5 435/6 435/69.1 435/DIG.14 435/DIG.15 435/DIG.2 435/DIG.3 435/DIG.4 436/501. G01N033/50 C12Q001/02.

☐ 26. 6312683 . 27 Jan 99; 06 Nov 01. Equine infectious anemia virus vectors. Kingsman; Alan John, et al. 424/93.2; 424/93.1 424/93.21 424/93.6 435/320.1 435/325 435/455 435/69.1 514/44 536/23.1. A61K048/00 C12N015/00 C12N015/88.

☐ 27. 6265390 . 22 Feb 99; 24 Jul 01. Methods for expressing nucleic acid sequences using nucleic acid constructs comprising hypoxia response elements. Ratcliffe; Peter John, et al. 514/44; 424/93.21 435/320.1 435/325 435/455 435/69.1. A01N043/04 A61K031/70 C12N015/00 C12N015/63.

☐ 28. 6218179 . 23 Jun 97; 17 Apr 01. Tissue specific hypoxia regulated constructs. Webster; Keith A., et al. 435/320.1; 435/325 435/455 536/23.1 536/24.1. C12N015/85.

☐ 29. 6207648 . 17 Jul 98; 27 Mar 01. Methods of using cytochrome P450 reductase for the enhancement of P450-based anti-cancer gene therapy. Waxman; David J., et al. 514/44; 435/320.1 435/455 536/23.2 536/23.4. A01N043/04 C12N015/00 C12N015/63 C07H021/04.

☐ 30. 5952226 . 09 May 97; 14 Sep 99. Hypoxia responsive EPO producing cells. Aebischer; Patrick, et al. 435/354; 435/325 435/366. C12N025/85.

☑ 31. 5942434 . 12 Dec 96; 24 Aug 99. Nucleic acid constructs comprising hypoxia response elements. Ratcliffe; Peter John, et al. 435/320.1; 424/93.21 435/325 435/455 435/69.1 435/69.3 435/69.8 514/44 536/23.5. C12N015/00 C12N015/63 C07H021/04 A01N043/04.

☑ 32. 5834306 . 23 Dec 94; 10 Nov 98. Tissue specific hypoxia regulated therapeutic constructs. Webster; Keith A., et al. 435/320.1; 536/24.1. C12N015/00 C07H021/04.

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(FILE 'HOME' ENTERED AT 15:21:02 ON 28 JAN 2003)

FILE 'MEDLINE, CAPLUS, BIOSIS, SCISEARCH' ENTERED AT 15:21:42 ON 28 JAN 2003

L1 2024 S HYPOXIA(W)RESPONSE(W)ELEMENT OR HRE
L2 22 S (HYPOXIA-INDUCIBLE(W)FACTOR OR HIF)(6A)(CONSENSUS(W)BIND?(W)S
L3 6327 S (SV40 OR MLV)(6A)(PROMOTER OR PROMOTOR)
L4 29 S L1 AND L3
L5 9 S L1 AND L2
L6 14 DUP REM L4 (15 DUPLICATES REMOVED)
L7 3 DUP REM L5 (6 DUPLICATES REMOVED)

=> d au ti so ab 1-14 l6

L6 ANSWER 1 OF 14 MEDLINE
AU Kietzmann Thomas; Samoylenko Anatoly; Roth Ulrike; Jungermann Kurt
TI Hypoxia-inducible factor-1 and **hypoxia response elements** mediate the induction of plasminogen activator inhibitor-1 gene expression by insulin in primary rat hepatocytes.
SO BLOOD, (2003 Feb 1) 101 (3) 907-14.
Journal code: 7603509. ISSN: 0006-4971.
AB The expression of the plasminogen activator inhibitor-1 (PAI-1) gene is enhanced by insulin both in vivo and in various cell types. Because insulin exerts a number of its biologic activities via the phosphatidylinositol 3-kinase and protein kinase B (PI3K/PKB) signaling pathway, it was the aim of the present study to investigate the role of the PI3K/PKB pathway in the expression of the PAI-1 gene and to identify the insulin responsive promoter sequences. It was shown that the induction of PAI-1 mRNA and protein expression by insulin and mild hypoxia could be repressed by the PI3K inhibitor wortmannin. Overexpression of a constitutively active PKB led to induction of PAI-1 mRNA expression and of luciferase (Luc) activity from a gene construct containing 766 bp of the rat PAI-1 promoter. Mutation of the **hypoxia response elements** (**HRE**-1 and **HRE**-2) in rat PAI-1 promoter, which could bind hypoxia inducible factor-1 (HIF-1), abolished the induction of PAI-1 by insulin and PKB. Insulin and the constitutive active PKB also induced Luc expression in cells transfected with the pGl3EPO-**HRE** Luc construct, containing 3 copies of the **HRE** from the erythropoietin gene in front of the **SV40 promoter**. Furthermore, insulin and the active PKB enhanced all 3 HIF alpha-subunit protein levels and HIF-1 DNA-binding activity, as shown by electrophoretic mobility shift assays (EMSAs). Thus, the insulin-dependent activation of the PAI-1 gene expression can be mediated via the PI3K/PKB pathway and the transcription factor HIF-1 binding to the **HREs** in the PAI-1 gene promoter.

L6 ANSWER 2 OF 14 CAPLUS COPYRIGHT 2003 ACS
IN Rapp, Jeffrey C.
TI Use of avian lysozyme promoter for transgenic human interferon .alpha.2b and monoclonal antibody synthesis in oviduct cells
SO PCT Int. Appl., 88 pp.
CODEN: PIXXD2
AB The present invention demonstrates the use of an avian lysozyme promoter in transgenic human interferon .alpha.2b (gene IFNMAGMAX) and monoclonal antibody synthesis in oviduct cells. The isolated nucleic acid of the present invention is useful for reducing the chromosomal positional effect of a transgene operably linked to the lysozyme gene expression control region and transfected into a recipient cell and allows expression of an operably linked heterologous nucleic acid insert in a transfected avian cells such as, for example, an oviduct cell. The isolated avian lysozyme of the present invention may be operably linked with a selected nucleic acid insert encoding a polypeptide desired to be expressed in a

transfected cell. The recombinant DNA of the present invention may further comprise a polyadenylation signal sequence or a chicken lysozyme 3' domain.

L6 ANSWER 3 OF 14 MEDLINE DUPLICATE 1
AU Reddy Ramachandra K; Dubeau Louis; Kleiner Heather; Parr Tyler; Nichols Peter; Ko Bryce; Dong Dezheng; Ko Howard; Mao Changhui; DiGiovanni John; Lee Amy S
TI Cancer-inducible transgene expression by the Grp94 promoter: spontaneous activation in tumors of various origins and cancer-associated macrophages.
SO CANCER RESEARCH, (2002 Dec 15) 62 (24) 7207-12.
Journal code: 2984705R. ISSN: 0008-5472.
AB A major challenge in treating cancer is the difficulty of bringing therapy to poorly perfused areas of solid tumors, which are often most resistant to chemotherapy and radiation. GRP94 is a chaperone protein localized in the endoplasmic reticulum with antiapoptotic properties. We report here that in vitro the proximal murine grp94 promoter is regulated differently from the **hypoxia response element** fused to the **SV40** minimal **promoter**, with glucose starvation as an inducer of grp94 but a potent repressor of the **hypoxia response element/SV40** fusion **promoter**. Through the use of transgenic mouse models, we showed that LacZ transgene expression driven by the grp94 promoter was strongly activated not only in spontaneous but also in a variety of chemically induced tumors. We additionally discovered that macrophages in the vicinity of malignant tumor showed a high level of transgene expression, consistent with intense beta-galactosidase staining at boundaries between viable tumor cells and necrotic areas. Isolated macrophages also showed grp94 mRNA and transgene activation under glucose starvation in vitro. In contrast, transgene activity was not detected in the normal tissue counterparts of any of the malignant tumors examined or macrophages associated with normal organs. These studies provide direct evidence that the tumor microenvironment is a potent physiological inducer of the grp94 promoter. The unique properties of the grp94 promoter suggest that it may offer a novel tool for directing transcription of therapeutic agents to tumors particularly in resistant regions bordering necrotic areas, delivered through standard vectors or macrophages.

L6 ANSWER 4 OF 14 CAPLUS COPYRIGHT 2003 ACS DUPLICATE 2
AU Farrell, Michael L.; Mertz, Janet E.
TI Cell type-specific replication of simian virus 40 conferred by hormone response elements in the late promoter
SO Journal of Virology (2002), 76(13), 6762-6770
CODEN: JOVIAM; ISSN: 0022-538X
AB The late genes of SV40 are not expressed at significant levels until after the onset of viral DNA replication. We previously identified two hormone response elements (**HREs**) in the late promoter that contribute to this delay. Mutants defective in these **HREs** overexpress late RNA at early, but not late, times after transfection of CV-1PD cells. Overexpression of nuclear receptors (NRs) that recognize these **HREs** leads to repression of the late promoter in a sequence-specific and titratable manner, resulting in a delay in late gene expression. These observations led to a model in which the late promoter is repressed at early times after infection by NRs, with this repression being relieved by titrn. of these repressors through simian virus 40 (SV40) genome replication to high copy no. Here, we tested this model in the context of the viral life cycle. SV40 genomes contg. mutations in either or both **HREs** that significantly reduce NR binding without altering the coding of any proteins were constructed. Competition for replication between mutant and wild-type viruses in low-multiplicity coinfections indicated that the +1 **HRE** offered a significant selective advantage to the virus within a few cycles of infection in African green monkey kidney cell lines CV-1, CV-1P, TC-7, MA-134, and Vero but not in CV-1PD' cells. Interestingly, the +55 **HRE** offered a

selective disadvantage in MA-134 cells but had no effect in CV-1, CV-1P, TC-7, Vero, and CV-1PD' cells. Thus, we conclude that these **HREs** are biol. important to the virus, but in a cell type-specific manner.

L6 ANSWER 5 OF 14 MEDLINE DUPLICATE 3
AU Farrell Michael L; Mertz Janet E
TI Hormone response element in **SV40** late **promoter** directly affects synthesis of early as well as late viral RNAs.
SO VIROLOGY, (2002 Jun 5) 297 (2) 307-18.
Journal code: 0110674. ISSN: 0042-6822.
AB We previously demonstrated that the presence of a hormone response element surrounding the transcription initiation site of the **SV40** major late **promoter** (+1 **HRE**) confers a replication advantage to the virus in a cell-type-specific manner. We determine here the mechanism by which the +1 **HRE** confers this advantage by analyzing in detail the various stages of the viral life cycle of wild-type versus a +1 **HRE** mutant in MA-134 cells. We show that the mutant overexpresses late genes at the expense of early genes at early times after infection. This initial underproduction of early RNA leads, subsequently, to an underproduction of large T-antigen, viral DNA, and infectious virions. We conclude that the +1 **HRE** is necessary for the proper initial regulation of transcription from the early as well as late promoter so the cascade of subsequent events can be executed for the optimal production of virions.

L6 ANSWER 6 OF 14 CAPLUS COPYRIGHT 2003 ACS
IN Webster, Keith A.
TI Combinations of silencer and inducible regulatory elements for tight regulation and strong induction of foreign genes in animal cells
SO PCT Int. Appl., 48 pp.
CODEN: PIXXD2
AB Expression vectors are disclosed that are comprised of one or more silencer elements and conditionally inducible elements to form silencer-inducible regions and promoters in operative linkage upstream of at least one expressed region. The expression vector thereby regulates expression of at least one downstream region by conditional silencing in which an expressed DNA region of a gene is transcribed. Use of multiple copies of the silencer lowers the basal level of expression of the gene and therefore increases the induction ratio. Genetically engineered mammalian cells and non-human mammals can be made using such expression vectors through transfection and transgenesis techniques. Moreover, processes of making and using the aforementioned products are disclosed (e.g., the expression vector may be used diagnostically, therapeutically, or prophylactically). A series of constructs using repeats of the silencer element (SIL) of the human synapsin gene and the **hypoxia response element** (**HRE**) of the phosphoglycerate kinase gene were prepd. and used to regulate expression of a luciferase reporter gene from the **SV40** early **promoter** in animal cells. Induction of the reporter gene in hypoxic skeletal myocytes was directly proportional to the no. of copies of SIL/**HRE** pairs in the promoter region. The construct was more effective in skeletal myocytes than in cardiac myocytes. In a rat ischemic hindlimb model induction ratios for the reporter gene under ischemic (hypoxic) conditions was >20 for constructs carrying three copies of the SIL/**HRE** pairs. For animals carrying only three copies of the **HRE** element and no silence elements the induction ratio was .apprx.1.4.

L6 ANSWER 7 OF 14 MEDLINE DUPLICATE 4
AU Krones A; Jungermann K; Kietzmann T
TI Cross-talk between the signals hypoxia and glucose at the glucose response element of the L-type pyruvate kinase gene.
SO ENDOCRINOLOGY, (2001 Jun) 142 (6) 2707-18.
Journal code: 0375040. ISSN: 0013-7227.
AB The signals oxygen and glucose play an important role in metabolism,

angiogenesis, tumorigenesis, and embryonic development. Little is known about an interaction of these two signals. We demonstrate here the cross-talk between oxygen and glucose in the regulation of L-type pyruvate kinase (L-PK) gene expression in the liver. In the liver the periportal to perivenous drop in O(2) tension was proposed to be an endocrine key regulator for the zonated gene expression. In primary rat hepatocyte cultures the expression of the L-PK gene on mRNA and on protein level was induced by venous pO(2), whereas its glucose-dependent induction occurred predominantly under arterial pO(2). It was shown by transient transfection of L-PK promoter luciferase and glucose response element (Glc(PK)RE) **SV40 promoter** luciferase gene constructs that the modulation by O(2) of the glucose-dependent induction occurred at the Glc(PK)RE in the L-PK gene promoter. The reduction of the glucose-dependent induction of the L-PK gene expression under venous pO(2) appeared to be mediated via an interference between hypoxia inducible factor-1 (HIF-1) and upstream stimulating factor at the Glc(PK)RE. The glucose response element also functioned as an **hypoxia response element** which was confirmed in cotransfection assays with Glc(PK)RE luciferase gene constructs and HIF-1alpha expression vectors. Furthermore, it was found by gel shift and supershift assay that HIF-1alpha and USF-1 or USF-2 could bind to the Glc(PK)RE. Our findings implicate that the cross-talk between oxygen and glucose might have a fundamental role in the regulation of several physiological and pathophysiological processes.

L6 ANSWER 8 OF 14 CAPLUS COPYRIGHT 2003 ACS
IN Binley, Katie Mary; Naylor, Stuart
TI A hypoxia-responsive regulatory element and its use in the expression of therapeutic genes in chronically hypoxic solid tumors
SO PCT Int. Appl., 154 pp.
CODEN: PIXXD2
AB A polynucleotide is provided which comprises at least two repeats of a **hypoxia response element** (**HRE**), wherein the hypoxia-inducible factor consensus binding sites within each of the two repeats are sepd. by a spacer of at least 20 contiguous nucleotides. A polynucleotide is also provides which comprised at least three repeats of a phosphoglycerate kinase (PGK) **hypoxia response element** (**HRE**) operably linked to an **SV40 promoter** or an **MLV promoter**. The polynucleotide may be operably linked to a nucleic acid of interest to drive expression under hypoxic conditions. In particular, the element can be used to drive expression of therapeutic genes in solid tumors as they are often undervascularized and chronically hypoxic. A series of **HREs** from a no. of different hypoxia-inducible genes were tested for hypoxia inducibility and those showing the strongest induction were selected for further characterization and optimization. The construct derived from the PGK gene, showing induction ratios of .gtoreq.100, was found to contain a consensus binding sequence for hypoxia-inducible factor (HIF) and these constructs could confer hypoxia inducible expression on a gene in macrophages. Although the construct contained the HIF-responsive element, no HIF could be detected, but macrophages manuf. the closely related EPAS-1 (endothelial PAS domain protein 1) transcription factor. The **HRE** could be used in combination with retroviral promoters. Gene therapy constructs using the **HRE** in combination with therapeutic genes, e.g. for a prodrug-activating cytochrome P 450, or tissue-specific regulatory elements such as interferon response elements are described.

L6 ANSWER 9 OF 14 CAPLUS COPYRIGHT 2003 ACS
AU Boast, Kate; Binley, Katie; Iqball, Sharifah; Price, Toby; Spearman, Hayley; Kingsman, Susan; Kingsman, Alan; Naylor, Stuart
TI Characterization of physiologically regulated vectors for the treatment of ischemic disease
SO Human Gene Therapy (1999), 10(13), 2197-2208

CODEN: HGTHE3; ISSN: 1043-0342

AB A high therapeutic index is as important for gene-based therapies as it is for chemotherapy or radiotherapy. One approach has been transcriptional targeting through the use of tissue-specific regulatory elements. A more versatile approach would be to use a regulatory element that is controlled via a parameter common to a broad range of diseases. Ischemia is characteristic of a no. of pathologies that range from vascular occlusion through to cancer. The state of low oxygen, hypoxia, triggers a transcriptional signaling pathway that is mediated by transcription factors binding to a specific enhancer, the **hypoxia response element** (**HRE**). These observations have therefore led to the use of **HREs** to drive gene expression in a no. of target tissues from tumors to cardiac muscle. To translate these observations into a clin. useful vector system we have now assessed the potency of a no. of naturally derived **HREs** in various configurations combined with minimal promoters. The optimal **HRE** has been introduced into a single transcription unit retroviral vector that can deliver regulated gene expression in response to hypoxia. An important feature of this new physiol. regulated vector is the combination of low basal expression and high-level activated expression that is on a par with that obtained with the cytomegalovirus immediate-early (CMV IE) promoter. The role of elements that stabilize mRNA in the presence of hypoxia has also been assessed. These hypoxia-regulated vectors may have utility for restricting the delivery of therapeutic proteins to tumors and ischemic sites.

L6 ANSWER 10 OF 14 MEDLINE DUPLICATE 5

AU Graven K K; Yu Q; Pan D; Roncarati J S; Farber H W

TI Identification of an oxygen responsive enhancer element in the glyceraldehyde-3-phosphate dehydrogenase gene.

SO BIOCHIMICA ET BIOPHYSICA ACTA, (1999 Oct 28) 1447 (2-3) 208-18. Journal code: 0217513. ISSN: 0006-3002.

AB The glycolytic enzyme glyceraldehyde-3-phosphate dehydrogenase (GAPDH) is induced by hypoxia in endothelial cells (EC). Upregulation occurs primarily at the level of transcription and occurs to a much greater extent in EC than in other cell types. To characterize EC specific **hypoxia response elements** within the GAPDH gene, we performed transient transfection studies in EC, fibroblasts and smooth muscle cells using portions of the GAPDH promoter linked to a CAT reporter gene. These initial studies identified an EC specific hypoxia responsive region that was further characterized (using **SV40-promoter**-CAT reporter constructs) as a 19-nucleotide sequence (-130 to -112) containing both an hypoxia inducible factor-1 (HIF-1)-binding site and a novel flanking sequence. Electrophoretic mobility shift assays confirmed inducible EC protein binding to this fragment. Mutation of either the HIF-1-binding site or the flanking sequence resulted in complete loss of function and loss of inducible protein binding. Thus, a single HIF-1-binding site is necessary, but not sufficient, for hypoxic regulation of GAPDH in EC. Furthermore, the novel HIF-1 flanking sequence required for GAPDH upregulation and the protein(s) that bind to it may be EC specific.

L6 ANSWER 11 OF 14 CAPLUS COPYRIGHT 2003 ACS

AU Stroup, Diane; Crestani, Maurizio; Chiang, John Y. L.

TI Orphan receptors chicken ovalbumin upstream promoter transcription factor II (COUP-TFII) and retinoid X receptor (RXR) activate and bind the rat cholesterol 7.alpha.-hydroxylase gene (CYP7A)

SO Journal of Biological Chemistry (1997), 272(15), 9833-9839 CODEN: JBCHA3; ISSN: 0021-9258

AB The cholesterol 7.alpha.-hydroxylase gene (CYP7A) is transcriptionally regulated by a no. of factors, including hormones, bile acids, and diurnal rhythm. Previous studies have identified a region from nucleotides (nt) -74 to -55 of the rat CYP7A promoter that enhanced bile acid repression of the **SV40** early **promoter**, as assayed with a luciferase

reporter gene in transiently transfected HepG2 cells. The rat CYP7A promoter/reporter activity was strongly stimulated by cotransfection with an expression plasmid encoding the nuclear hormone receptor chicken ovalbumin upstream promoter transcription factor II (COUP-TFII) in a dose-dependent manner. Site-directed mutagenesis in the region of nt -74 to -55 altered this stimulation. Recombinant COUP-TFII expressed in HepG2 or COS-1 cells were found to bind to nt -74 -55 and nt -149 -128 probes by electrophoretic mobility shift assay (EMSA) and by supershifting the corresponding band with COUP-TFII-specific antibodies. The region of nt -176 -117 was previously mapped as a retinoic acid response region and was found to bind retinoid X receptor (RXR). EMSA supershift assays of wild-type and mutant oligomers using antibody against RXR revealed that the sequences between nt -145 and -134 were important for RXR binding. We conclude that COUP-TFII stimulates the transcriptional activity of the rat CYP7A promoter by binding to the sequences between nt -74 to -54 and nt -149 to -128. RXR may stimulate CYP7A gene transcription by binding to a direct repeat of the hormone response element sepd. by one nucleotide located at nt -146 -134.

L6 ANSWER 12 OF 14 CAPLUS COPYRIGHT 2003 ACS
AU Crestani, Maurizio; Sadeghpour, Azita; Stroup, Diane; Galli, Giovanni; Chiang, John Y. L.
TI The opposing effects of retinoic acid and phorbol esters converge to a common response element in the promoter of the rat cholesterol 7.alpha.-hydroxylase gene (CYP7A)
SO Biochemical and Biophysical Research Communications (1996), 225(2), 585-592
CODEN: BBRCA9; ISSN: 0006-291X
AB The activity of the rat CYP7A/luciferase reporter gene was increased five-fold by all-trans retinoic acid (atRA) or 9-cis retinoic acid (9cRA) in transient transfection assay in HepG2 cells. Cotransfection with retinoid X receptor (RXR) stimulated the promoter activity in the absence of ligand, however, addn. of atRA inhibited the transcriptional activity. Cotransfection with retinoic acid receptor (RAR) did not have much effect on CYP7A promoter activity. The CYP7A **promoter**, when linked upstream to the **SV40**/luciferase reporter gene, strongly repressed the phorbol 12-myristate 13-acetate (PMA)-stimulated SV40/luciferase reporter gene activity. The regions conferring the effects of RA and PMA were mapped to nt - 176/-117 and nt -148/-129, resp. Several direct repeats of hormone response element (AGTTCA) in this region are required for RA response. AP-1 like sequences are located within the region responding to both RA and PMA. Site-directed mutagenesis of the AP-1 site abolished the effects of both RA and phorbol esters. Retinoic acid effect was antagonized by PMA. Moreover, cotransfection of Fos and Jun expression vectors blunted the stimulatory effect of retinoic acid on the CYP7 gene transcription in the liver.

L6 ANSWER 13 OF 14 MEDLINE DUPLICATE 6
AU Lin T M; Young W J; Chang C
TI Multiple functions of the TR2-11 orphan receptor in modulating activation of two key cis-acting elements involved in the retinoic acid signal transduction system.
SO JOURNAL OF BIOLOGICAL CHEMISTRY, (1995 Dec 15) 270 (50) 30121-8. Journal code: 2985121R. ISSN: 0021-9258.
AB The testicular receptor 2 (TR2) orphan receptor binds to hormone response elements (**HREs**) consisting of two AGGTCA half-site direct repeat consensus sequences (DR) with various spacing in the following order: DR1 > DR2 > DR5 DR4 DR6 > DR3. When binding to natural **HREs**, TR2 orphan receptor remains flexible with higher binding affinities to (a) cellular retinol-binding protein II **promoter** region (CRBPIIp) (DR1), **SV40** +55 region (DR2), and retinoic acid response element beta (RARE beta) (DR5) than to (b) NGFI-B response element (NBRE) and also to (c) the palindromic thyroid hormone response element (TREpal). This wide spectrum of **HRE** recognition sequences suggests possible

versatility of the TR2 orphan receptor in cross-talking with other signal transduction systems. Chloramphenicol acetyltransferase (CAT) assay demonstrates that the TR2 orphan receptor competes with CRBPIIp- and RARE beta-CAT gene expression activated by retinoid X receptor alpha (RXR alpha) and retinoic acid receptor alpha (RAR alpha)/RXR alpha heterodimers, respectively. In addition, this suppression may not be mediated by the formation of heterodimers between TR2 orphan receptor and either RXR alpha or RAR alpha. Instead, a minimum of 100-fold higher affinity of the TR2 orphan receptor for CRBPIIp than RXR alpha may explain why the TR2 orphan receptor dominates RXR alpha in CRBPIIp-CAT activation. Together, our data suggest that the TR2 orphan receptor may be a master regulator in modulating the activation of two key **HREs**, RARE beta and CRBPIIp, involved in the retinoic acid signal transduction pathway.

L6 ANSWER 14 OF 14 BIOSIS COPYRIGHT 2003 BIOLOGICAL ABSTRACTS INC.
AU Webster, Keith A. (1); Wu, Xiaosu (1); Prentice, Howard; Hicks, Martin N.; McDonald, Patricia; Wylie, Andrew; Discher, Daryl J. (1); Bishopric, Nanette H. (1)
TI Hypoxia regulated vectors for targeting genes to ischemic myocardium.
SO Circulation, (1995) Vol. 92, No. 8 SUPPL., pp. I756.
Meeting Info.: 68th Scientific Session of the American Heart Association Anaheim, California, USA November 13-16, 1995
ISSN: 0009-7322.

=> d au ti so ab 1-3 l7

L7 ANSWER 1 OF 3 MEDLINE DUPLICATE 1
AU Grosfeld Alexandra; Andre Jocelyne; Hauguel-De Mouzon Sylvie; Berra Edurne; Pouyssegur Jacques; Guerre-Millo Michele
TI Hypoxia-inducible factor 1 transactivates the human leptin gene promoter.
SO JOURNAL OF BIOLOGICAL CHEMISTRY, (2002 Nov 8) 277 (45) 42953-7.
Journal code: 2985121R. ISSN: 0021-9258.
AB Increased placental leptin has been demonstrated in preeclampsia, a pregnancy disorder associated with placental hypoxia. This suggests that leptin gene expression is enhanced in response to oxygen deficiency in this organ. In support of this hypothesis, we have previously shown that hypoxia activates the leptin promoter in trophoblast-derived BeWo cells. Hypoxia-inducible factor 1 (HIF-1) is a heterodimeric HIF-1alpha/HIF-1beta complex that regulates the transcription of hypoxia-responsive genes. To test whether this factor is involved in hypoxia-induced leptin promoter activation, BeWo cells were transiently transfected with a HIF-1alpha expression vector. Exogenous HIF-1alpha markedly increased luciferase reporter activity driven by the leptin promoter when HIF-1beta was co-expressed in the same cells. This effect was similar to that elicited by CoCl2, an agent known to stabilize endogenous HIF-1alpha. These data suggest that HIF-1alpha/HIF-1beta dimers are involved in the effect of CoCl2 to activate the leptin promoter. To confirm the implication of HIF-1, the cells were transfected with a dominant negative form of HIF-1alpha producing transcriptionally inactive HIF-1beta/HIF-1alpha dimers. This mutant HIF-1alpha protein abolished CoCl2 activation of the leptin promoter, providing direct evidence that the effect of CoCl2 is mediated by endogenous HIF-1alpha. Deletion analysis and site-specific mutagenesis demonstrated that a **HIF**-1 **consensus binding site** (**HRE**) spanning -120 to -116 bp relative to the start site was required for CoCl2 and exogenous HIF-1alpha induction of leptin promoter activity. Electrophoretic mobility shift assays performed with in vitro-translated HIF-1alpha and HIF-1beta proteins demonstrated binding to this **HRE** and not to mutated sequences only when both subunits were used together. These data demonstrate that leptin is a new hypoxia-inducible gene, which is stimulated in a placental cell line through HIF-1 interaction with a consensus **HRE** site located at -116 in the proximal promoter.

L7 ANSWER 2 OF 3 MEDLINE DUPLICATE 2
AU Lu Shan; Gu Xiang; Hoestje Sara; Epner Daniel E
TI Identification of an additional hypoxia responsive element in the glyceraldehyde-3-phosphate dehydrogenase gene promoter.
SO BIOCHIMICA ET BIOPHYSICA ACTA, (2002 Mar 19) 1574 (2) 152-6.
Journal code: 0217513. ISSN: 0006-3002.
AB Glyceraldehyde-3-phosphate dehydrogenase (GAPDH) is a multifunctional enzyme overexpressed in many tumors and induced by hypoxia in normal and malignant cells. The degree to which hypoxia transcriptionally activates GAPDH is cell type specific. The GAPDH promoter region contains a hypoxia responsive element (**HRE**) consisting of a **hypoxia inducible factor**-1 (**HIF**-1) **consensus binding site** plus adjacent sequence [Graven et al. (1999) Biochim. Biophys. Acta 1447, 208-218]. Using transient transfection experiments with the GAPDH promoter region linked to a luciferase reporter gene, we found that GAPDH was transcriptionally activated by hypoxia in each of three human prostate cancer cell lines tested, with the greatest level of induction in the most differentiated cell line. Using sequence analysis of the GAPDH promoter region, we identified a novel **HRE** distinct from the previously characterized one that consists of two consensus HIF-1 sites arranged as inverted repeats separated by 5 bp. Hypoxia transcriptionally activated a promoter construct in which the previously characterized **HRE** was mutated and the novel **HRE** remained intact. Heterologous promoter constructs containing only one or two copies of the novel **HRE** plus a minimal promoter consisting of a TATA box drove hypoxia inducible expression of the luciferase reporter gene in transient transfection assays. Mutation of HIF-1 sites within the novel **HRE** resulted in complete loss of function.

L7 ANSWER 3 OF 3 CAPLUS COPYRIGHT 2003 ACS
IN Binley, Katie Mary; Naylor, Stuart
TI A hypoxia-responsive regulatory element and its use in the expression of therapeutic genes in chronically hypoxic solid tumors
SO PCT Int. Appl., 154 pp.
CODEN: PIXXD2
AB A polynucleotide is provided which comprises at least two repeats of a **hypoxia response element** (**HRE**), wherein the **hypoxia-inducible factor consensus binding sites** within each of the two repeats are sepd. by a spacer of at least 20 contiguous nucleotides. A polynucleotide is also provides which comprised at least three repeats of a phosphoglycerate kinase (PGK) **hypoxia response element** (**HRE**) operably linked to an SV40 promoter or an MLV promoter. The polynucleotide may be operably linked to a nucleic acid of interest to drive expression under hypoxic conditions. In particular, the element can be used to drive expression of therapeutic genes in solid tumors as they are often undervascularized and chronically hypoxic. A series of **HREs** from a no. of different hypoxia-inducible genes were tested for hypoxia inducibility and those showing the strongest induction were selected for further characterization and optimization. The construct derived from the PGK gene, showing induction ratios of .gtoreq.100, was found to contain a consensus binding sequence for hypoxia-inducible factor (HIF) and these constructs could confer hypoxia inducible expression on a gene in macrophages. Although the construct contained the HIF-responsive element, no HIF could be detected, but macrophages manuf. the closely related EPAS-1 (endothelial PAS domain protein 1) transcription factor. The **HRE** could be used in combination with retroviral promoters. Gene therapy constructs using the **HRE** in combination with therapeutic genes, e.g. for a prodrug-activating cytochrome P 450, or tissue-specific regulatory elements such as interferon response elements are described.

=> d bib 3 l7

L7 ANSWER 3 OF 3 CAPLUS COPYRIGHT 2003 ACS
AN 2000:210397 CAPLUS
DN 132:247196
TI A hypoxia-responsive regulatory element and its use in the expression of therapeutic genes in chronically hypoxic solid tumors
IN Binley, Katie Mary; Naylor, Stuart
PA Oxford Biomedica (UK) Limited, UK
SO PCT Int. Appl., 154 pp.
CODEN: PIXXD2
DT Patent
LA English
FAN.CNT 3

	PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
PI	WO 2000017371	A1	20000330	WO 1999-GB3181	19990922
	W: AE, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CU, CZ, DE, DK, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, UA, UG, US, UZ, VN, YU, ZA, ZW, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM				
	RW: GH, GM, KE, LS, MW, SD, SL, SZ, TZ, UG, ZW, AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG				
	WO 9915684	A2	19990401	WO 1998-GB2885	19980923
	WO 9915684	A3	19990610		
	W: AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CU, CZ, DE, DK, EE, ES, FI, GB, GE, GH, GM, HR, HU, ID, IL, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, UA, UG, US, UZ, VN, YU, ZW, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM				
	RW: GH, GM, KE, LS, MW, SD, SZ, UG, ZW, AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG				
	CA 2343324	AA	20000330	CA 1999-2343324	19990922
	AU 9962130	A1	20000410	AU 1999-62130	19990922
	EP 1115877	A1	20010718	EP 1999-949142	19990922
	R: AT, BE, CH, DE, DK, ES, FR, GB, GR, IT, LI, LU, NL, SE, MC, PT, IE, SI, LT, LV, FI, RO				
	JP 2002526083	T2	20020820	JP 2000-574270	19990922
PRAI	WO 1998-GB2885	W	19980923		
	GB 1999-1906	A	19990128		
	GB 1999-3538	A	19990216		
	GB 1997-20216	A	19970923		
	GB 1997-20465	A	19970925		
	WO 1999-GB3181	W	19990922		

RE.CNT 11 THERE ARE 11 CITED REFERENCES AVAILABLE FOR THIS RECORD
ALL CITATIONS AVAILABLE IN THE RE FORMAT

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=> d his

(FILE 'HOME' ENTERED AT 15:21:02 ON 28 JAN 2003)

FILE 'MEDLINE, CAPLUS, BIOSIS, SCISEARCH' ENTERED AT 15:21:42 ON 28 JAN 2003

L1 2024 S HYPOXIA(W)RESPONSE(W)ELEMENT OR HRE
L2 22 S (HYPOXIA-INDUCIBLE(W)FACTOR OR HIF)(6A)(CONSENSUS(W)BIND?(W)S
L3 6327 S (SV40 OR MLV)(6A)(PROMOTER OR PROMOTOR)
L4 29 S L1 AND L3
L5 9 S L1 AND L2
L6 14 DUP REM L4 (15 DUPLICATES REMOVED)
L7 3 DUP REM L5 (6 DUPLICATES REMOVED)
L8 415 S HYPOXIA(W)RESPONSE(W)ELEMENT
L9 213 S L8 AND (PROMOTER OR REGULATORY(W)(ELEMENT OR SEQUENCE))
L10 14013 S (VIRAL OR SV40 OR CMV OR MMLV OR MLV)(3A)PROMOTER
L11 32 S L8 AND L10
L12 11 DUP REM L11 (21 DUPLICATES REMOVED)

=> d bib ab 1-11 l12

L12 ANSWER 1 OF 11 MEDLINE
AN 2003022730 IN-PROCESS
DN 22417176 PubMed ID: 12393531
TI Hypoxia-inducible factor-1 and **hypoxia response elements** mediate the induction of plasminogen activator inhibitor-1 gene expression by insulin in primary rat hepatocytes.
AU Kietzmann Thomas; Samoylenko Anatoly; Roth Ulrike; Jungermann Kurt
CS Institut fur Biochemie und Molekulare Zellbiologie, Gottingen, Germany.
SO BLOOD, (2003 Feb 1) 101 (3) 907-14.
Journal code: 7603509. ISSN: 0006-4971.
CY United States
DT Journal; Article; (JOURNAL ARTICLE)
LA English
FS IN-PROCESS; NONINDEXED; Abridged Index Medicus Journals; Priority Journals
ED Entered STN: 20030117
Last Updated on STN: 20030117
AB The expression of the plasminogen activator inhibitor-1 (PAI-1) gene is enhanced by insulin both in vivo and in various cell types. Because insulin exerts a number of its biologic activities via the phosphatidylinositol 3-kinase and protein kinase B (PI3K/PKB) signaling pathway, it was the aim of the present study to investigate the role of the PI3K/PKB pathway in the expression of the PAI-1 gene and to identify the insulin responsive promoter sequences. It was shown that the induction of PAI-1 mRNA and protein expression by insulin and mild hypoxia could be repressed by the PI3K inhibitor wortmannin. Overexpression of a constitutively active PKB led to induction of PAI-1 mRNA expression and of luciferase (Luc) activity from a gene construct containing 766 bp of the rat PAI-1 promoter. Mutation of the **hypoxia response elements** (HRE-1 and HRE-2) in rat PAI-1 promoter, which could bind hypoxia inducible factor-1 (HIF-1), abolished the induction of PAI-1 by insulin and PKB. Insulin and the constitutive active PKB also induced Luc expression in cells transfected with the pGl3EPO-HRE Luc construct, containing 3 copies of the HRE from the erythropoietin gene in front of the **SV40 promoter**. Furthermore, insulin and the active PKB enhanced all 3 HIF alpha-subunit protein levels and HIF-1 DNA-binding activity, as shown by electrophoretic mobility shift assays (EMSAs). Thus, the insulin-dependent activation of the PAI-1 gene expression can be mediated via the PI3K/PKB pathway and the transcription factor HIF-1 binding to the HREs in the PAI-1 gene promoter.

L12 ANSWER 2 OF 11 MEDLINE DUPLICATE 1
AN 2002738327 IN-PROCESS

DN 22387069 PubMed ID: 12499260
TI Cancer-inducible transgene expression by the Grp94 promoter: spontaneous activation in tumors of various origins and cancer-associated macrophages.
AU Reddy Ramachandra K; Dubeau Louis; Kleiner Heather; Parr Tyler; Nichols Peter; Ko Bryce; Dong Dezheng; Ko Howard; Mao Changhui; DiGiovanni John; Lee Amy S
CS Department of Biochemistry and Molecular Biology, University of Southern California/Norris Comprehensive Cancer Center, University of Southern California Keck School of Medicine, Los Angeles, California 90089-9176, USA.
NC CA27607 (NCI)
CA59318 (NCI)
CA79750 (NCI)
SO CANCER RESEARCH, (2002 Dec 15) 62 (24) 7207-12.
Journal code: 2984705R. ISSN: 0008-5472.
CY United States
DT Journal; Article; (JOURNAL ARTICLE)
LA English
FS IN-PROCESS; NONINDEXED; Priority Journals
ED Entered STN: 20021228
Last Updated on STN: 20021228
AB A major challenge in treating cancer is the difficulty of bringing therapy to poorly perfused areas of solid tumors, which are often most resistant to chemotherapy and radiation. GRP94 is a chaperone protein localized in the endoplasmic reticulum with antiapoptotic properties. We report here that in vitro the proximal murine grp94 promoter is regulated differently from the **hypoxia response element** fused to the **SV40** minimal **promoter**, with glucose starvation as an inducer of grp94 but a potent repressor of the **hypoxia response element/SV40** fusion **promoter**. Through the use of transgenic mouse models, we showed that LacZ transgene expression driven by the grp94 promoter was strongly activated not only in spontaneous but also in a variety of chemically induced tumors. We additionally discovered that macrophages in the vicinity of malignant tumor showed a high level of transgene expression, consistent with intense beta-galactosidase staining at boundaries between viable tumor cells and necrotic areas. Isolated macrophages also showed grp94 mRNA and transgene activation under glucose starvation in vitro. In contrast, transgene activity was not detected in the normal tissue counterparts of any of the malignant tumors examined or macrophages associated with normal organs. These studies provide direct evidence that the tumor microenvironment is a potent physiological inducer of the grp94 promoter. The unique properties of the grp94 promoter suggest that it may offer a novel tool for directing transcription of therapeutic agents to tumors particularly in resistant regions bordering necrotic areas, delivered through standard vectors or macrophages.

L12 ANSWER 3 OF 11 MEDLINE DUPLICATE 2
AN 2002496193 MEDLINE
DN 22224368 PubMed ID: 12239150
TI Long-term reversal of chronic anemia using a hypoxia-regulated erythropoietin gene therapy.
AU Binley Katie; Askham Zoe; Iqball Sharifah; Spearman Hayley; Martin Leigh; de Alwis Mahesh; Thrasher Adrian J; Ali Robin R; Maxwell Patrick H; Kingsman Susan; Naylor Stuart
CS Oxford BioMedica (UK) Ltd; Molecular Immunology Unit, Institute of Child Health, London, United Kingdom.. k.binley@oxfordbiomedica.co.uk
SO BLOOD, (2002 Oct 1) 100 (7) 2406-13.
Journal code: 7603509. ISSN: 0006-4971.
CY United States
DT Journal; Article; (JOURNAL ARTICLE)
LA English
FS Abridged Index Medicus Journals; Priority Journals
EM 200212

ED Entered STN: 20021003
Last Updated on STN: 20021217
Entered Medline: 20021205

AB Anemia is a common clinical problem, and there is much interest in its role in promoting left ventricular hypertrophy through increasing cardiac workload. Normally, red blood cell production is adjusted through the regulation of erythropoietin (Epo) production by the kidney. One important cause of anemia is relative deficiency of Epo, which occurs in most types of renal disease. Clinically, this can be corrected by supplementation with recombinant Epo. Here we describe an oxygen-regulated gene therapy approach to treating homozygous erythropoietin-SV40 T antigen (Epo-TAg(h)) mice with relative erythropoietin deficiency. We used vectors in which murine Epo expression was directed by an Oxford Biomedica **hypoxia response element** (OBHRE) or a constitutive cytomegalovirus (**CMV**) **promoter**. Both corrected anemia, but CMV-Epo-treated mice acquired fatal polycythemia. In contrast, OBHRE-Epo corrected the hematocrit level in anemic mice to a normal physiologic level that stabilized without resulting in polycythemia. Importantly, the OBHRE-Epo vector had no significant effect on the hematocrit of control mice. Homozygous Epo-TAg(h) mice display cardiac hypertrophy, a common adaptive response in patients with chronic anemia. In the OBHRE-Epo-treated Epo-TAg(h) mice, we observed a significant reversal of cardiac hypertrophy. We conclude that the OBHRE promoter gives rise to physiologically regulated Epo secretion such that the hematocrit level is corrected to healthy in anemic Epo-TAg(h) mice. This establishes that a hypoxia regulatory mechanism similar to the natural mechanism can be achieved, and it makes EPO gene therapy more attractive and safer in clinical settings. We envisage that this control system will allow regulated delivery of therapeutic gene products in other ischemic settings.

L12 ANSWER 4 OF 11 MEDLINE DUPLICATE 3
AN 2002154268 MEDLINE
DN 21877094 PubMed ID: 11882625
TI Vigilant vector: heart-specific promoter in an adeno-associated virus vector for cardioprotection.
AU Phillips M Ian; Tang Yi; Schmidt-Ott Kai; Qian Keping; Kagiyama Shuntaro
CS Department of Physiology and Functional Genomics, University of Florida, Gainesville, FL 32610-0274, USA.. MIP@ufl.edu
NC HL 27339 (NHLBI)
SO HYPERTENSION, (2002 Feb) 39 (2 Pt 2) 651-5.
Journal code: 7906255. ISSN: 1524-4563.
CY United States
DT Journal; Article; (JOURNAL ARTICLE)
LA English
FS Priority Journals
EM 200203
ED Entered STN: 20020312
Last Updated on STN: 20020403
Entered Medline: 20020328

AB Repeated bouts of ischemia in the heart lead to fibrosis and eventually to heart failure. Although certain genes, such as SOD or hemoxygenase and antisense to AT(1)R, ACE, and (beta(1)-AR can provide short-term protection of the heart from ischemia, there is no known mechanism for constantly responding to repeated incidences of ischemia. We hypothesized that a "vigilant vector," designed to be expressed specifically in the heart and switch on therapeutic genes only during hypoxia, would provide cardioprotection. To attain cardiac specificity, we inserted an MLC2v promoter into an adeno-associated virus (AAV) designed to deliver AS to AT(1)R and gfp. In in vitro experiments in cardiomyocytes (H9C2 cells), the MLC2v-AAV-gfp drove gene expression in all cells at levels comparable to a cytomegalovirus (**CMV**) **promoter**. In in vivo experiments, the rAAV-MLC2v-gfp was injected intravenously into mice or rats. Green fluorescence protein (GFP) DNA was located in kidney, heart

(right and left ventricle), lung, adrenal and spleen. GFP mRNA, however, was expressed only in the heart and absent in other tissues. To switch on the rAAV transgene during ischemia, we inserted a **hypoxia response element** (HRE). This upregulates transcription when O(2) levels are low. Thus, there are 4 components to the vigilant vector; a gene switch (HRE), a heart-specific promoter (MLC2v), a therapeutic gene (AS-AT(1)R) and a reporter gene (gfp). To silence or lower basal level of expression while retaining specificity, we have reduced the length of the MLC2v promoter from 3 kb to 1775 bp or 281 bp. The truncated promoter is equally effective in heart specific expression. Preliminary studies with the rAAV-HRE-gfp in vitro show an increased expression in 1% O(2) in 4 to 6 hours. By adding additional hypoxia-inducible factor (HIFalpha) (5 microg), the MLC2v-gfp expression is increased by 4-fold in 1% O(2). Further amplification of the gene to 400-fold in 1% O(2) can be achieved with a double plasmid. The construct may serve as a prototype "vigilant vector" to switch on therapeutic genes in specific tissue with physiological signals.

L12 ANSWER 5 OF 11 CAPLUS COPYRIGHT 2003 ACS
AN 2001:489616 CAPLUS
DN 135:88021
TI Combinations of silencer and inducible regulatory elements for tight regulation and strong induction of foreign genes in animal cells
IN Webster, Keith A.
PA University of Miami, USA
SO PCT Int. Appl., 48 pp.
CODEN: PIXXD2
DT Patent
LA English
FAN.CNT 1

	PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
PI	WO 2001048187	A2	20010705	WO 2000-US33269	20001215
	WO 2001048187	A3	20020530		
	WO 2001048187	C2	20021107		
	W: CA, JP				
	RW: AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, TR				
	EP 1242592	A2	20020925	EP 2000-984041	20001215
	R: AT, BE, CH, DE, DK, ES, FR, GB, GR, IT, LI, LU, NL, SE, MC, PT, IE, SI, LT, LV, FI, RO, MK, CY, AL, TR				
PRAI	US 1999-171597P	P	19991223		
	US 2000-723326	A	20001128		
	WO 2000-US33269	W	20001215		

AB Expression vectors are disclosed that are comprised of one or more silencer elements and conditionally inducible elements to form silencer-inducible regions and promoters in operative linkage upstream of at least one expressed region. The expression vector thereby regulates expression of at least one downstream region by conditional silencing in which an expressed DNA region of a gene is transcribed. Use of multiple copies of the silencer lowers the basal level of expression of the gene and therefore increases the induction ratio. Genetically engineered mammalian cells and non-human mammals can be made using such expression vectors through transfection and transgenesis techniques. Moreover, processes of making and using the aforementioned products are disclosed (e.g., the expression vector may be used diagnostically, therapeutically, or prophylactically). A series of constructs using repeats of the silencer element (SIL) of the human synapsin gene and the **hypoxia response element** (HRE) of the phosphoglycerate kinase gene were prepd. and used to regulate expression of a luciferase reporter gene from the **SV40** early **promoter** in animal cells. Induction of the reporter gene in hypoxic skeletal myocytes was directly proportional to the no. of copies of SIL/HRE pairs in the promoter region. The construct was more effective in skeletal myocytes than in cardiac

myocytes. In a rat ischemic hindlimb model induction ratios for the reporter gene under ischemic (hypoxic) conditions was >20 for constructs carrying three copies of the SIL/HRE pairs. For animals carrying only three copies of the HRE element and no silence elements the induction ratio was .apprx.1.4.

L12 ANSWER 6 OF 11 MEDLINE DUPLICATE 4
AN 2001264398 MEDLINE
DN 21255656 PubMed ID: 11356723
TI Cross-talk between the signals hypoxia and glucose at the glucose response element of the L-type pyruvate kinase gene.
AU Krones A; Jungermann K; Kietzmann T
CS Institut fur Biochemie und Molekulare Zellbiologie, Georg-August-Universitat, Humboldtallee 23, D-37073 Gottingen, Germany.
SO ENDOCRINOLOGY, (2001 Jun) 142 (6) 2707-18.
Journal code: 0375040. ISSN: 0013-7227.
CY United States
DT Journal; Article; (JOURNAL ARTICLE)
LA English
FS Abridged Index Medicus Journals; Priority Journals
EM 200106
ED Entered STN: 20010625
Last Updated on STN: 20010625
Entered Medline: 20010621
AB The signals oxygen and glucose play an important role in metabolism, angiogenesis, tumorigenesis, and embryonic development. Little is known about an interaction of these two signals. We demonstrate here the cross-talk between oxygen and glucose in the regulation of L-type pyruvate kinase (L-PK) gene expression in the liver. In the liver the periportal to perivenous drop in O(2) tension was proposed to be an endocrine key regulator for the zonated gene expression. In primary rat hepatocyte cultures the expression of the L-PK gene on mRNA and on protein level was induced by venous pO(2), whereas its glucose-dependent induction occurred predominantly under arterial pO(2). It was shown by transient transfection of L-PK promoter luciferase and glucose response element (Glc(PK)RE) **SV40 promoter** luciferase gene constructs that the modulation by O(2) of the glucose-dependent induction occurred at the Glc(PK)RE in the L-PK gene promoter. The reduction of the glucose-dependent induction of the L-PK gene expression under venous pO(2) appeared to be mediated via an interference between hypoxia inducible factor-1 (HIF-1) and upstream stimulating factor at the Glc(PK)RE. The glucose response element also functioned as an **hypoxia response element** which was confirmed in cotransfection assays with Glc(PK)RE luciferase gene constructs and HIF-1alpha expression vectors. Furthermore, it was found by gel shift and supershift assay that HIF-1alpha and USF-1 or USF-2 could bind to the Glc(PK)RE. Our findings implicate that the cross-talk between oxygen and glucose might have a fundamental role in the regulation of several physiological and pathophysiological processes.

L12 ANSWER 7 OF 11 CAPLUS COPYRIGHT 2003 ACS
AN 2000:210397 CAPLUS
DN 132:247196
TI A hypoxia-responsive regulatory element and its use in the expression of therapeutic genes in chronically hypoxic solid tumors
IN Binley, Katie Mary; Naylor, Stuart
PA Oxford Biomedica (UK) Limited, UK
SO PCT Int. Appl., 154 pp.
CODEN: PIXXD2
DT Patent
LA English
FAN.CNT 3

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE

PI WO 2000017371 A1 20000330 WO 1999-GB3181 19990922
W: AE, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CU, CZ, DE, DK, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, UA, UG, US, UZ, VN, YU, ZA, ZW, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM
RW: GH, GM, KE, LS, MW, SD, SL, SZ, TZ, UG, ZW, AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG
WO 9915684 A2 19990401 WO 1998-GB2885 19980923
WO 9915684 A3 19990610
W: AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CU, CZ, DE, DK, EE, ES, FI, GB, GE, GH, GM, HR, HU, ID, IL, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, UA, UG, US, UZ, VN, YU, ZW, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM
RW: GH, GM, KE, LS, MW, SD, SZ, UG, ZW, AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG
CA 2343324 AA 20000330 CA 1999-2343324 19990922
AU 9962130 A1 20000410 AU 1999-62130 19990922
EP 1115877 A1 20010718 EP 1999-949142 19990922
R: AT, BE, CH, DE, DK, ES, FR, GB, GR, IT, LI, LU, NL, SE, MC, PT, IE, SI, LT, LV, FI, RO
JP 2002526083 T2 20020820 JP 2000-574270 19990922
PRAI WO 1998-GB2885 W 19980923
GB 1999-1906 A 19990128
GB 1999-3538 A 19990216
GB 1997-20216 A 19970923
GB 1997-20465 A 19970925
WO 1999-GB3181 W 19990922

AB A polynucleotide is provided which comprises at least two repeats of a **hypoxia response element** (HRE), wherein the hypoxia-inducible factor consensus binding sites within each of the two repeats are sepd. by a spacer of at least 20 contiguous nucleotides. A polynucleotide is also provides which comprised at least three repeats of a phosphoglycerate kinase (PGK) **hypoxia response element** (HRE) operably linked to an **SV40 promoter** or an **MLV promoter**. The polynucleotide may be operably linked to a nucleic acid of interest to drive expression under hypoxic conditions. In particular, the element can be used to drive expression of therapeutic genes in solid tumors as they are often undervascularized and chronically hypoxic. A series of HREs from a no. of different hypoxia-inducible genes were tested for hypoxia inducibility and those showing the strongest induction were selected for further characterization and optimization. The construct derived from the PGK gene, showing induction ratios of .gtoreq.100, was found to contain a consensus binding sequence for hypoxia-inducible factor (HIF) and these constructs could confer hypoxia inducible expression on a gene in macrophages. Although the construct contained the HIF-responsive element, no HIF could be detected, but macrophages manuf. the closely related EPAS-1 (endothelial PAS domain protein 1) transcription factor. The HRE could be used in combination with retroviral promoters. Gene therapy constructs using the HRE in combination with therapeutic genes, e.g. for a prodrug-activating cytochrome P 450, or tissue-specific regulatory elements such as interferon response elements are described.

RE.CNT 11 THERE ARE 11 CITED REFERENCES AVAILABLE FOR THIS RECORD
ALL CITATIONS AVAILABLE IN THE RE FORMAT

L12 ANSWER 8 OF 11 MEDLINE DUPLICATE 5
AN 1999426539 MEDLINE
DN 99426539 PubMed ID: 10498251
TI Characterization of physiologically regulated vectors for the treatment of

ischemic disease.
AU Boast K; Binley K; Iqball S; Price T; Spearman H; Kingsman S; Kingsman A; Naylor S
CS Biochemistry Department, Oxford University, UK.
SO HUMAN GENE THERAPY, (1999 Sep 1) 10 (13) 2197-208.
Journal code: 9008950. ISSN: 1043-0342.
CY United States
DT Journal; Article; (JOURNAL ARTICLE)
LA English
FS Priority Journals
EM 199910
ED Entered STN: 20000111
Last Updated on STN: 20000111
Entered Medline: 19991027
AB A high therapeutic index is as important for gene-based therapies as it is for chemotherapy or radiotherapy. One approach has been transcriptional targeting through the use of tissue-specific regulatory elements. A more versatile approach would be to use a regulatory element that is controlled via a parameter common to a broad range of diseases. Ischemia is characteristic of a number of pathologies that range from vascular occlusion through to cancer. The state of low oxygen, hypoxia, triggers a transcriptional signaling pathway that is mediated by transcription factors binding to a specific enhancer, the **hypoxia response element** (HRE). These observations have therefore led to the use of HREs to drive gene expression in a number of target tissues from tumors to cardiac muscle. To translate these observations into a clinically useful vector system we have now assessed the potency of a number of naturally derived HREs in various configurations combined with minimal promoters. The optimal HRE has been introduced into a single transcription unit retroviral vector that can deliver regulated gene expression in response to hypoxia. An important feature of this new physiologically regulated vector is the combination of low basal expression and high-level activated expression that is on a par with that obtained with the cytomegalovirus immediate-early (**CMV** IE) **promoter**. The role of elements that stabilize mRNA in the presence of hypoxia has also been assessed. These hypoxia-regulated vectors may have utility for restricting the delivery of therapeutic proteins to tumors and ischemic sites.

L12 ANSWER 9 OF 11 MEDLINE DUPLICATE 6
AN 1999448026 MEDLINE
DN 99448026 PubMed ID: 10516721
TI An adenoviral vector regulated by hypoxia for the treatment of ischaemic disease and cancer.
AU Binley K; Iqball S; Kingsman A; Kingsman S; Naylor S
CS Oxford BioMedica (UK) Ltd, Medawar Centre, Oxford Science Park, Oxford, UK.
SO GENE THERAPY, (1999 Oct) 6 (10) 1721-7.
Journal code: 9421525. ISSN: 0969-7128.
CY ENGLAND: United Kingdom
DT Journal; Article; (JOURNAL ARTICLE)
LA English
FS Priority Journals
EM 200004
ED Entered STN: 20000427
Last Updated on STN: 20000427
Entered Medline: 20000420
AB Recombinant adenoviral vectors have a number of advantages for gene therapy, including transduction of a range of dividing and non-dividing cell types. However, this broad range may be a disadvantage if non-target cells are transduced and are adversely affected by expression of the transferred gene. Here we describe a novel adenoviral vector in which transcription of the transgene is restricted to the patho-physiological condition of low oxygen tension (hypoxia). Hypoxia activates the

expression of a number of genes, principally via the stabilisation of members of the bHLH/PAS family of transcription factors that bind to a con- sensus DNA sequence, the **hypoxia response element** (HRE). We have configured an optimised HRE expression cassette into an adenoviral vector, AdOBHRE. A range of cell types, including primary human skeletal muscle, when transduced with AdOBHRE display a low basal level of transgene expression that is highly induced in hypoxia to levels equivalent to that obtained from the **CMV promoter**. The AdOBHRE vector could be exploited for transcriptionally targeted gene therapy for the treatment of diseases such as cancer, peripheral arterial disease, arthritis and anaemia where tissue hypoxia is a cardinal feature.

L12 ANSWER 10 OF 11 MEDLINE DUPLICATE 7
AN 2000011278 MEDLINE
DN 20011278 PubMed ID: 10542317
TI Identification of an oxygen responsive enhancer element in the glyceraldehyde-3-phosphate dehydrogenase gene.
AU Graven K K; Yu Q; Pan D; Roncarati J S; Farber H W
CS The Pulmonary Center, Boston University School of Medicine, 715 Albany Street, R-304, Boston, MA 02118, USA.. kkg@medicine.wisc.edu
NC HL-45537 (NHLBI)
HL03125-01 (NHLBI)
SO BIOCHIMICA ET BIOPHYSICA ACTA, (1999 Oct 28) 1447 (2-3) 208-18.
Journal code: 0217513. ISSN: 0006-3002.
CY Netherlands
DT Journal; Article; (JOURNAL ARTICLE)
LA English
FS Priority Journals
EM 199912
ED Entered STN: 20000113
Last Updated on STN: 20000113
Entered Medline: 19991208
AB The glycolytic enzyme glyceraldehyde-3-phosphate dehydrogenase (GAPDH) is induced by hypoxia in endothelial cells (EC). Upregulation occurs primarily at the level of transcription and occurs to a much greater extent in EC than in other cell types. To characterize EC specific **hypoxia response elements** within the GAPDH gene, we performed transient transfection studies in EC, fibroblasts and smooth muscle cells using portions of the GAPDH promoter linked to a CAT reporter gene. These initial studies identified an EC specific hypoxia responsive region that was further characterized (using **SV40-promoter**-CAT reporter constructs) as a 19-nucleotide sequence (-130 to -112) containing both an hypoxia inducible factor-1 (HIF-1)-binding site and a novel flanking sequence. Electrophoretic mobility shift assays confirmed inducible EC protein binding to this fragment. Mutation of either the HIF-1-binding site or the flanking sequence resulted in complete loss of function and loss of inducible protein binding. Thus, a single HIF-1-binding site is necessary, but not sufficient, for hypoxic regulation of GAPDH in EC. Furthermore, the novel HIF-1 flanking sequence required for GAPDH upregulation and the protein(s) that bind to it may be EC specific.

L12 ANSWER 11 OF 11 BIOSIS COPYRIGHT 2003 BIOLOGICAL ABSTRACTS INC.
AN 1996:13963 BIOSIS
DN PREV199698586098
TI Hypoxia regulated vectors for targeting genes to ischemic myocardium.
AU Webster, Keith A. (1); Wu, Xiaosu (1); Prentice, Howard; Hicks, Martin N.; McDonald, Patricia; Wylie, Andrew; Discher, Daryl J. (1); Bishopric, Nanette H. (1)
CS (1) Molecular Cardiol. Lab., SRI International, Menlo Park, CA USA
SO Circulation, (1995) Vol. 92, No. 8 SUPPL., pp. I756.
Meeting Info.: 68th Scientific Session of the American Heart Association Anaheim, California, USA November 13-16, 1995

ISSN: 0009-7322.
DT Conference
LA English

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